

## Role of *N*-Acetylgalactosamine-Specific Protein in Trapping of Nematodes by *Arthrobotrys oligospora*

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An *N*-acetylgalactosamine-specific protein was purified from mycelial homogenates of the nematode-trapping fungus *Arthrobotrys oligospora* by using affinity chromatography. The molecular weight of the protein was estimated at 22,000 by its comparative mobility on sodium dodecyl sulfate-polyacrylamide slab gels. Pretreatment of nematodes with the purified protein reduced entrapment, indicating a role for the sugar-binding protein in recognition and capture of prey by the fungus.

Nematode-trapping fungi are hyphomycetes that capture prey by using special hyphal structures which are adhesive or constrictive. *Arthrobotrys oligospora* Fresn. traps nematodes in adhesive mycelial networks (2), and recognition by the fungus of its prey has been attributed to a molecular interaction of certain proteins on the fungal surface with sugar molecules comprising the nematode cuticle (3-5). However, all evidence for the existence of fungal protein that binds sugar is indirect. It consists solely of demonstrations that exposure of hyphal traps to specific sugars interferes with nematode capture (5, 7). It was desirable, therefore, to attempt direct isolation from the fungus of a protein able to bind specific cuticular sugar molecules and influence the process of nematode capture.

The strain of *A. oligospora* employed throughout the present investigation was demonstrated to respond, as reported by others elsewhere (5), to treatment with *N*-acetyl-D-galactosamine (GalNAc). Fungal mycelia immersed in a 20 mM solution of the sugar for 24 h displayed a decreased capacity to trap nematodes, which was estimated at 35 to 50% of that of untreated controls.

*A. oligospora* was maintained in the laboratory by transfer at 3- to 4-week intervals on cornmeal agar (Difco Laboratories, Detroit, Mich.). It was grown in quantity on a filtered extract of cornmeal prepared by boiling 17 g of commercial ground grain in 1 liter of distilled water and processing through filter paper (Whatman no. 1). The extract was dispensed in 300-ml quantities per 1-liter flask and autoclaved for 20 min at 121°C. Each flask was inoculated with an *A. oligospora* spore suspension and incubated on a reciprocating shaker for 14 days at 28°C. The mycelial mats were collected by filtration, combined, washed repeatedly with sterile distilled water, macerated in a Potter-Elvehjem homogenizer with 3 volumes of cold 0.1 M phosphate buffer (phosphate-buffered saline [PBS]) (pH 7.2) containing 0.15 M NaCl and 1 mM of the protease inhibitor phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 18,000 rpm for 45 min, and the supernatant was analyzed by affinity chromatography (6) at 0 to 4°C with a 4-ml column of Affi-gel galactosamine (Bio-Rad Laboratories, Richmond, Calif.) with specificity for GalNAc-binding proteins. A 25- to 30-ml quantity of mycelial extract was added to the column, and the column was washed with PBS (0.1 M, pH 7.2) containing

1.0 M NaCl until measurement of absorbance at 280 nm indicated that most of the protein was eluted. The column was then washed with PBS to remove the high-salt buffer. Sugar-specific proteins that were bound to the column were eluted with PBS (0.1 M, pH 7.2) containing 0.3 M lactose. The protein concentration of the eluate was monitored by absorbance at 280 nm, as well as by dye-binding assay (Bio-Rad). The elution profile illustrated by Fig. 1 indicates that bound protein was effectively separated from protein that lacked affinity for the adsorbant.

The protein fraction that was eluted with lactose-containing buffer was dialyzed repeatedly against 250 volumes of PBS (0.1 M, pH 7.2) and lyophilized to a powder which was stored at -20°C. Homogeneity of the product was determined by sodium dodecyl sulfate-slab gel electrophoresis. Gels were developed as described by Ansorge (1), by fixation in a methanol solution of copper chloride and trichloroacetic acid and by treatment first with 0.1% silver nitrate and then with a solution of 2.0% potassium carbonate in 0.01% formaldehyde. The isolated protein proved homogeneous and displayed a molecular weight estimated at 22,000 by comparison of its mobility on polyacrylamide gels with that of the following molecular weight markers: bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,000), and lactalbumin (14,400).

The purified GalNAc-specific fungal protein was examined also for an effect on entrapment of nematodes by *A. oligospora*. For this purpose, the free-living nematode *Panagrellus silusiae* was cultured axenically in a chemically defined medium as described by Rothstein (8). The nematodes were collected by centrifugation, washed several times with sterile distilled water, and immersed for 20 min at 28°C in a PBS (0.1 M, pH 7.2) solution of the purified, GalNAc-specific fungal protein at a concentration of 100 µg/ml. Nematodes were immersed also in commercially available GalNAc-specific protein from *Glycine max* (Sigma Chemical Co., St. Louis, Mo.) and in PBS (0.1 M, pH 7.2) as controls. After treatment, nematodes were washed thoroughly with PBS and placed on the surface of cornmeal agar in petri dishes inoculated 7 days previously with *A. oligospora*. Each dish received ca. 30 nematodes, and the influence of treatment on their entrapment by the fungus was observed microscopically after 24 and 48 h. The results recorded in Table 1 clearly indicate that nematodes treated with the purified GalNAc-specific fungal protein were not trapped as readily as untreated nematodes, presumably because recog-

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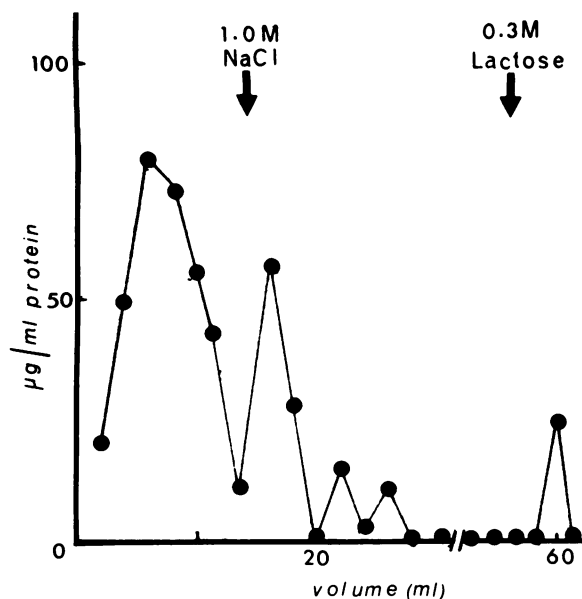


FIG. 1. Affinity chromatography of mycelial homogenates of *A. oligospora* on an Affi-gel galactosamine column.

niton and capture of nematodes by the fungus was interfered with at the molecular level.

The results reported here complement those of others (5, 7), who observed that nematode capture is inhibited by various sugars and suggested that entrapment is mediated by interaction of a lectin on the surface of the fungus trap and a specific sugar on the nematode cuticle. The presence of GalNAc residues on the nematode cuticle has already been demonstrated (5, 9). The GalNAc-specific protein isolated by affinity chromatography from homogenates of *A. oligospora* mycelium displayed binding characteristics ascribed to lectins, and it may have a role in the recognition and capture of nematodes by the fungus. However, if the activity of nematode-trapping fungi is directed by reactions with the specificity of lectins, the fungi would be expected to be selective in their choice of prey. But that is not the case. They are indiscriminant. A single fungus species can trap many different species of nematodes and even other animals of microscopic dimensions (2). Nematode-trapping fungi may depend on lectin reactions for recognition of prey, but it is doubtful that lectins can account fully for the broad spectrum of activity and the remarkable tenacity of the

TABLE 1. Effect of treatment of nematodes on their entrapment by *A. oligospora*

Treatment	No. of nematodes <sup>a</sup> at:			
	24 h		48 h	
	Free	Trapped	Free	Trapped
Phosphate buffer	15	15	2	27
<i>Glycine max</i> , GalNAc-specific lectin	24	17	9	26
Purified protein from <i>A. oligospora</i>	22	7	13	17

<sup>a</sup> Average of three separate experiments.

mucilage produced by *A. oligospora* and other species that capture nematodes by adhesion. The broad specificity also could be mediated by a lectin that specifically recognizes sugar residues common to many or all nematodes.

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